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THE BEHAVIOR OF THE CHROMOSOMES IN *OENOTHERA LATA*×*O. GIGAS*

OCNTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **128**

REGINALD RUGGLES GATES

(WITH PLATES XII–XIV)

The hybrid which forms the subject of this paper is of peculiar interest because one of its parents has double the number of chromosomes possessed by the other, *O. lata* having usually 14 chromosomes and *O. gigas* 28. Very few cases of this sort are known, either in plants or animals. But a further complication arises in the fact that *O. gigas* is known to have originated from *O. Lamarckiana*, which also has 14 chromosomes, and *O. gigas* has in all probability attained the tetraploid number by a duplication of the chromosome set present in *O. Lamarckiana*.[1] Then if fertilization took place in the ordinary manner, the hybrid *O. lata*×*O. gigas* would be expected to have 21 chromosomes, 7 derived from the *lata* egg and 14 (which is probably a double set of *Lamarckiana* chromosomes) derived from the male cell of *gigas*. Under these circumstances, the behavior of the chromosomes in the hybrid, especially during the period of reduction and germ-cell formation, is a matter of especial interest.

The general results regarding chromosome numbers and distribution were obtained some time ago, and a brief statement was published (**10**), but the cytological evidence is here presented for the first time. Many of the drawings for these figures were completed about two years ago, but my interest in other phases of this work has postponed their publication until now. The earlier stages of reduction in Oenothera, up to the end of the heterotypic mitosis, have already been described in detail (**11**), so that this paper will deal chiefly with the later stages, beginning with the metaphase of the heterotypic mitosis.

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[1] I have discussed this matter in another paper to appear in the *Archiv für Zellforschung*. DEVRIES in a footnote to a recent paper (5) considers it an important question whether the doubling in *O. gigas* is accomplished through a longitudinal or a transverse splitting. In all the cases, of which I am aware, of chromosome multiplication under experimental conditions without nuclear division (BOVERI 4, LILLIE 23), the chromosomes evidently divide longitudinally as in ordinary somatic mitoses so that it seems probable that the division in *O. gigas* has also been longitudinal (on the very probable assumption that it occurred after fertilization). This view is further supported by the fact that the chromosomes of *O. gigas* show no apparent change in size or shape from those of *O. Lamarckiana*. If the division had been transverse, such a change might perhaps be expected. It is not impossible that in *Drosera longifolia*, which has 40 small chromosomes (ROSENBERG 31), while *D. rotundifolia* has 20 larger ones, there has been a transverse split of the *D. rotundifolia* chromosomes.

The plants from which these studies were made were grown at Wood's Hole, Mass., in 1905 and 1906, from seeds of DEVRIES. The results show that in some cases the number of chromosomes is undoubtedly 21, while in one individual it was 20.[2] The number is undoubtedly constant in an individual, however, as shown by a large number of counts, which demonstrated constantly 20 in one case and 21 in the other.

The external characters were not studied with sufficient care at that time to describe them accurately, but from my notes they appear to have been intermediate between *O. lata* and *O. gigas*. DEVRIES has described in a recent paper (**5**) the hybrids of *O. gigas* with other forms. I have called attention elsewhere to the fact that the behavior of *O. gigas* in hybridization, as well as its number of chromosomes, places it in a different category from the other mutants of *O. Lamarckiana*. DEVRIES finds that *O. gigas*×*O. Lamarckiana* forms a constant race intermediate between the parents, at least to the second hybrid generation. *O. gigas*×*O. Lamarckiana*, *O. Lamarckiana* ×*O. gigas*, *O. gigas*×*O. brevistylis*, *O. gigas*×*O. rubrinervis*, and *O. rubrinervis*×*O. gigas*, all give constant hybrid races which are externally alike in all these crosses. *O. lata*×*O. gigas*, however, gives in the F_1 two types, about 50 per cent. of each; type I intermediate between *O. lata* and *O. gigas*, type II intermediate between *O. Lamarckiana* and *O. gigas*. These presumably would all have about 21 chromosomes. There were 133 plants in the culture of this hybrid in 1907 and a smaller number in 1908.

Miss LUTZ (**24**) from a study of 40 individuals of *O. lata*×*O. gigas* finds more complex conditions in this cross, though apparently she

[2] In my first paper (**8**) this individual was thought to be *O. lata*×*O. Lamarckiana*, but was afterward found to be derived from fertilization by foreign *gigas* pollen, this particular seed package not having been guarded as was supposed when the seeds were planted.

has failed clearly to recognize the types included in her class III. She divides the offspring into three classes. Class I has the characters of pure *O. lata*, and the two individuals which appeared are each said to have 15 chromosomes.[3] Class II consists of *O. gigas* plants having about 30 chromosomes. There were six of these plants, and their presence cannot be accounted for by the ordinary methods of fertilization. If the *O. gigas* male cell united with two *O. lata* nuclei in the embryo sac, this would account for the origin of the *O. gigas* number of chromosomes. But GEERTS (**12, 13**) finds that the embryo sac of Oenothera contains only four nuclei, the egg, two synergids, and one polar nucleus, so that the possibilities here are more limited than in an 8-nucleate sac.[4] The class III of Miss LUTZ apparently includes both the types of DEVRIES'S cross, but they are not characterized so that a comparison can be made.

These interesting facts all show that there is still much to be explained regarding *O. gigas* and its hybrids. In the paper already referred to, I have shown that, in all the tissues examined, the cells are larger in *O. gigas* than in *O. Lamarckiana*, though the percentage of increase varies in different tissues. The production of the tetraploid number of chromosomes results in larger nuclei and larger cells, and this in turn in many cases produces larger organs. Certain changes which are found in the relative dimensions of the cells will account for the altered shape of certain organs. Thus the differences between *O. Lamarckiana* and *O. gigas* can probably be analyzed into two factors, (1) increased size of the cells, and (2) altered relative dimensions of the cells in certain cases. The former undoubtedly results directly from the doubling of the chromosome number; at least the double number of chromosomes and the larger size of cells occur simultaneously. Whether the latter is a necessary consequence upon the increase in cell size and a resulting change in cell relations, or is due to an independent factor, is uncertain. It should be said that characters, such as leaf shape, which might be accounted for by a change in the relative dimensions of the cells (though I have not as yet made measurements of the leaf cells to determine this), are extremely variable, and it seems not unlikely that this extreme variability may result from variation in cell dimensions consequent upon the readjustment to the double chromosome number.

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[3] The presence of pure *O. lata* in this cross strongly suggests apogamy in this mutant, and it seems not unlikely that a condition exists resembling in part that found by ROSENBERG (**32**) in *Hieracium excellens*, in which part of the embryo sacs are developed after normal reduction processes and require fertilization, while occasionally embryo sacs are produced without reduction (apogamously or aposporously), in which case the egg develops without fertilization. I hope to test this hypothesis experimentally this summer.

[4] Whether an individual with the tetraploid number of chromosomes would have the *O. gigas* characters, owing to its *number* of chromosomes rather than their *source*, is a question I have considered in a forthcoming paper (footnote 1). The view that the number is the main factor is supported, perhaps, by the fact that all the mutants of *O. Lamarckiana* experimented with (except *O. lata* which is peculiar in its crosses), when crossed with *O. gigas* give a constant hybrid of the same type in every case.

DESCRIPTION

The cytological account will begin with the telophase of the heterotypic mitosis in the pollen mother cell. The ten figures in plate XII are from the plant having 20 chromosomes, and were drawn on a smaller scale than those of the other two plates. At this stage the chromosomes can be counted with perfect accuracy. A large number of counts of this telophase show that 10 chromosomes enter each daughter nucleus. In a number of cases 10 were counted at each end of the same spindle. In several instances 11 were found in one of the daughter groups, and in a few cases it was possible to show that the corresponding daughter group contained only 9 chromosomes. In this plant, then, there are 20 chromosomes which segregate into two groups of 10 each in the reduction division, one chromosome occasionally going to the wrong group. Counts of somatic cells, made long ago in tissues of the anther, also showed that 20 chromosomes were present.

The material from which the figures in plate XII were drawn was subjected to an exceptionally high temperature in the process of imbedding, and in a few cases this has apparently affected the shape of the viscous chromosomes. *Figs. 1–5* are early telophases before the formation of a nuclear membrane. In *fig. 1* the chromosomes nearly all show their bivalent nature, and in most of them the two halves are dumb-bell shaped. This clubbing of the chromosomes at the ends is a common phenomenon both at this time and in the telophase of the homotypic and somatic mitoses. I have already referred to these appearances in an earlier paper (**9**, p. 19). In *fig. 2* the chromosomes are more irregular in shape, and their bivalent character is not so evident. There are 11 chromosomes in the daughter group, and examination of the adjacent sections showed that there were 9 and no more at the other end of the spindle. The globular black bodies seen in the figure are frequently found scattered near the periphery of the cytoplasm. They stain like chromatin with Haidenhain's iron alum hematoxylin, but their chemical nature is unknown. *Figs. 3–5* each show 10 chromosomes. *Fig. 6* is a telophase with 11 chromosomes, the next section showing 9 at the other end of the spindle. *Fig. 1* is an exact polar view, the other figures being more or less oblique cuts of the spindle, but the spindle fibers are not represented. *Figs. 7, 8* are somewhat later stages in side view, soon after the nuclear membrane is formed. Ten chromosomes are present in each. In *figs. 9, 10* are shown in outline the loop-shaped chromosomes of two somatic cells. Each has 20 chromosomes. The cells are from the middle layers of the anther wall.

Plates XIII and XIV deal with plants having 21 chromosomes. *Fig. 11* is a side view of the heterotypic spindle, showing 20 or 21 chromosomes. It is usually difficult to count the chromosomes exactly at this time in a side view of the spindle, on account of the close aggregation of some of them, and the presence of spindle fibers. The chromosomes are almost never regularly oriented in an equatorial plate on the heterotypic spindle. An examination of the literature shows that in most plants and animals a flat equatorial plate is formed in the metaphase of this mitosis as in other mitoses, although a few forms constitute exceptions. But the homotypic mitosis in Oenothera has always a very definite equatorial plate, in which the chromosomes are oriented in a single plane. There can be little doubt that the irregularities in chromosome distribution arise from this failure of the chromosomes to be regularly paired and oriented on the heterotypic spindle. The irregularities in distribution certainly arise at this time.

I have recently confirmed practically all the events of reduction by a study of the wild *O. biennis*, so that this account of reduction applies to the genus Oenothera in general and is not the result of mutative conditions. This will be referred to again later.

Fig. 12 is another cell in telophase. One nucleus is uncut, showing 10 bivalent chromosomes. The other was sectioned by the knife. *Fig. 13* shows 11 chromosomes and a number of very small nucleoli. Usually only one or two larger nucleoli are present. In *fig. 14* there are 12 bivalent chromosomes. In such cases 9 appear at the opposite end of the spindle. *Figs. 17, 18* are very early telophases just after the nuclear membrane has been formed. The daughter nuclei grow very quickly to the size of *figs. 19, 20*, each of which shows 11 bivalent chromosomes, while in the nucleus represented in *fig. 21* there are only 9. In general I have found that the nucleus having 9 chromosomes is likely to be appreciably smaller than one having 11. Evidently the amount of karyolymph secreted by a nucleus is a function of its number of chromosomes. This is also shown by the many cases of single chromosomes left behind in the cytoplasm and forming small nuclei during reduction, as observed in various forms, especially in hybrids.

These drawings of telophases are nearly all from nuclei which are uncut by the knife in sectioning. The sections are usually 10 μ thick, so that in a majority of cases the nucleus is completely contained in one section. The nuclear membrane is conspicuous; hence by focusing it can be determined with certainty that the membrane is uncut and that the contents of the nucleus are intact. All the nuclei in which counts have been made have been first shown to be intact in this manner. Moreover, whenever the chromosomes in both daughter nuclei could be counted, their sum was invariably found to be 21, whether 10+11 or, as occurred in a few cases, 9+12.

There are a number of interesting features about the telophase, which I have studied with particular care. In the first place it is in the best stage for counting the chromosomes with absolute accuracy, and is even better than diakinesis, because of the reduced number of chromosomes. In my last paper on reduction (**11**) I showed that pairing before the reduction division only takes place to a limited extent, and that the shapes characteristic of heterotypic chromosomes are therefore usually absent at this time, as will be seen from *figs. 26–34* of the paper referred to. During the period of interkinesis, however, the entire chromosomes of the heterotypic mitosis having split to form bivalents in which the two parts are closely held together, show the characteristic X, Y, V, H, K shapes, etc. (See *figs. 12–14, 19–21.*)

A large number of counts of the chromosomes were made in these telophases, and it was found that the numbers 10 and 11 occurred with approximately equal frequency, while the numbers 9 and 12 were only occasional. Every single case which was admitted as a count could be determined with absolute certainty as a case in which there were, for instance, just 10 chromosomes, no more and no less.

In the anaphase of the heterotypic mitosis, the chromosomes as they pass to the poles are nearly globular or somewhat elongated in shape, and are at first closely massed at the poles of the spindle. There is considerable variation in the time of appearance of the split in these chromosomes, though they usually appear bivalent in the early telophase. They appear to come into actual contact at this time, forming a compact group, but never fusing or uniting. They very soon begin to separate, however, and as they do so nuclear sap appears between and to a lesser extent around them. Then the nuclear membrane appears where the karyolymph comes in contact with the cytoplasm. The nucleus so formed is at first very small, but grows rapidly to its full size by the increase in nuclear sap.

LAWSON (**20**) has described this process in detail in *Passiflora coerulea.* He found that the chromosomes fused into a single mass in the telophase, and that the karyolymph begins to be secreted within this chromatic mass and later comes to surround it. The cytoplasm, coming in contact with the karyolymph, forms the nuclear membrane, which is therefore the limiting membrane of the cytoplasm, just as is the tonoplast of a vacuole. It is undoubtedly true that the nuclear membrane is formed where the karyolymph comes in contact with the cytoplasm, although it must remain uncertain whether the karyolymph is secreted by the chromosomes or merely attracted and accumulated about them from the cytoplasm. There must be extremely little if any cytoplasm included in the nucleus so formed, because the nuclear membrane appears close about the chromosome group when the chromosomes are still almost in contact. This supports GRÉGOIRE'S observations (**14, 15**), that the structural contents of the resting nucleus are formed wholly from the chromosomes, and there can be no doubt, in this case at least, that the daughter nucleus is formed entirely from the chromosomes and the karyolymph in which they float. This is to me a strong observational evidence of chromosome continuity.

Unlike LAWSON'S description for *Passiflora coerulea*, the chromosomes in Oenothera certainly do not fuse in the telophase of the heterotypic mitosis, but maintain their separate identity completely at first, and usually more or less completely thoughout the period of interkinesis. Occasionally stages are found which indicate that they string out and anastomose to some extent during a late stage of interkinesis, partly losing their sharp boundaries, but this stage apparently does not very often occur.

Nucleoli may be formed *de novo* in these daughter nuclei, as I have described previously (**8**, p. 93). The chromosomes in this telophase are all clearly bivalents, in which the halves are closely in contact. I have examined thousands of nuclei in this stage and have never seen the halves other than more or less closely in contact. On the other hand, it is universally true in my observations that no two chromosome bivalents are ever found in contact. Not only is this the case, but they are invariably distributed at approximately equal distances from each other, just within the nuclear membrane. I have never seen an exception to this in any Oenothera studied. The position of the chromosomes might be explained by supposing that they are attached to the nuclear membrane from the first, and are thus carried outward as the nucleus grows.[5] In many cases the chromosomes appear actually to be attached to the nuclear membrane, or at least to be lying very closely against it. This, however, leaves unexplained the fact that the chromosomes are never in contact with each other at this time, and further that they are very generally placed at approximately equal distances from each other, just within the nuclear wall. All these facts point to the supposition that the chromosome bivalents are mutually repelled. It is true that in the early telophase the chromosomes form a close group, so that they certainly cannot be repelled at that time, but may be attracted. However, the medium in which bodies float frequently changes their qualities of attraction and repulsion, and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float. The facts all suggest that the chromosome bivalents mutually repel each other at this time, while the halves of these are held together, probably by attraction.

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[5] It might also be supposed that in the dehydration processes preparatory to mounting, the chromosomes would be drawn against the nuclear membrane. But in such a case one would undoubtedly find the chromosomes occasionally massed on one side of the nucleus or irregularly placed, instead of being always at regular intervals about the periphery. From the regularity of their placing I have no doubt that the chromosomes occupy their original positions within the nucleus, and there is no indication that they are ever disturbed by the processes of fixation, imbedding, and staining, when properly carried out.

The studies of WILSON (37) and others on insect chromosomes show that there are *selective* attractions between certain chromosomes at the time of synapsis or at some other period of meiosis. These of course require something more specific than electromagnetic forces to explain them.

Fig. 22 shows one of the nuclei of a pollen mother cell in the prophase of the homotypic mitosis. Eleven chromosomes are present, having the same bivalent structure as in the telophase of the previous mitosis. Both nuclei always go through the various stages of the second mitosis simultaneously. The method of spindle formation has not been studied with great care, but corresponds with the Gladiolus type of multipolar spindle formation (LAWSON 19, 21). There is no indication of an intra-nuclear network, the spindle being wholly extra-nuclear in origin. A portion of the weft of fibers surrounding the nuclear membrane remains *in situ* when the latter disappears, forming a close meshwork, against which the chromosomes lie. The fibers then rearrange themselves; the fibrillae from the cones already formed come in and become attached to the chromosomes; finally the spindle becomes bipolar by the rearrangement of the fibers forming the cones.

The numerous papers on spindle formation in angiosperms need not be cited here. Among the more recent critical studies of this structure may be mentioned that of BERGHS (2), who insists that the distinction between kinoplasm and trophoplasm will not hold; that the spindle results simply from the gradual orientation of the material of the cytoplasmic reticulum, and returns to a reticulum after mitosis.

Sometimes spindle formation begins on one side of the nucleus, as in *fig. 23*. In such cases the cones of the multipolar spindle may be formed and the nuclear membrane may have disappeared on one side of the nucleus before any indication of spindle formation has appeared on the other side.

Fig. 15 is a polar view of the two homotypic spindles in metaphase. There are 11 chromosomes on one spindle and 10 on the other. *Fig. 25* is another at the same stage, showing 10 chromosomes. In *fig. 24* the spindles are at right angles and all the chromosomes are not shown. In the side view of the spindle some of the chromosomes appear like tetrads, owing to their bivalent structure. On the homotypic spindle, before the chromosomes divide, they are very regularly oriented in a single plane in the equatorial plate, as in *figs. 15, 25*. This contrasts strikingly with the heterotypic spindle, in which the chromosomes are scattered for a considerable distance along the long axis of the spindle, so that there is usually no metaphase, strictly speaking.

Fig. 26 is an early anaphase of the homotypic mitosis, showing one spindle in side view and one in polar view. In the polar view 20 chromosomes are found by focusing through a short distance, and the remaining 2 are found on the next section. In the side view the chromosomes could not all be counted, but presumably there were 20 after division. Two pale-staining nucleoli still persist in the cytoplasm.

Fig. 16 shows three of the nuclei in the telophase of the homotypic mitosis. Many of the chromosomes have a characteristic two-lobed or dumb-bell shape. One chromosome is left behind in the cytoplasm, leaving 10 chromosomes each in two of the daughter nuclei. This two-lobed shape is a characteristic appearance of the chromosomes in the telophase of somatic mitoses, but presumably bears no relation to the next mitosis, because if this were the beginning of a split for the next mitosis it would indicate that the division of the chromosomes is transverse. But metaphase and anaphase stages of somatic mitoses show that the chromosomes divide longitudinally. Of course the possibility that the transverse axis of a chromosome in telophase should regularly become its longitudinal axis before the next metaphase, is not excluded, although this seems unlikely.

The great difference in the size of the figures made it impossible to arrange them in order on the plates. The magnification is the same as in my last paper in this journal (**11**), so that the figures can be directly compared.

DISCUSSION

The history of any ontogeny is the history of the transformation of chemical metabolism into definite structures, or rather the eventuation of a series of chemical processes in the production of a series of physical structures. Morphologists and cytologists map the succession of structures appearing and call it a series of events. They are not unmindful, however, that the primary process is the metabolism, the structures its by-products, which in turn, so far as they are capable of continuing metabolism, produce other structures and, particularly in the adult organism, structures like themselves. Similarly, the chromosomes of the germ nuclei must be thought of as definite aggregations of chemical materials, which initiate or take part in certain forms of metabolism. The chromosomes themselves do not (at any rate not wholly) control their growth or division, but this is more or less subject to the conditions of temperature, etc., in which they are placed, as the work of ERDMANN (**7**) and others has shown. Similarly, as the complex of physical and chemical conditions in the nucleus and cell undergoes changes, the chromosomes change from the compact to the alveolate or distributed condition, or *vice versa*, etc. The cytologists who record these events are aware that chemical transformations are continually going on, and that the changes in visible physical structures are the external concomitant of such chemical processes.

Chemical reactions in the test tube, however complex, do not lead to the production of any structures more complex than crystals or flocculent masses of various sorts. There are indications that living matter frequently has the properties of liquid crystals, but why do the infinitely more complex reactions of living matter build structures of such relative permanency and of tremendous intricacy? The problem of individual development, from this standpoint, is the question, How do certain forms of chemical metabolism result in the production of certain visible physical structures or characters? Of course, our present microscopic appliances do not permit us to know how many structural steps, if any, there may be between the interacting molecular masses and the finest structures visible under our highest powers, but it is by no means necessary to assume that there are such. This digression will indicate to some extent, perhaps, the viewpoint of the writer in connection with the cytological aspects of this work.

No attempt will be made to discuss here the literature of reduction, only a very few of the recent papers being referred to. The discussion of matters of cytological detail will be taken up at another time.

One important matter, which was discussed in a former paper (**11**), concerns the method of chromosome reduction, i. e., whether there is a pairing of threads about the time of synizesis and whether the spirem afterward breaks into a single series or two parallel series of chromosomes. In the paper referred to I established the fact (as will be conceded, I think, after a study of *figs. 20–32*, particularly, of that paper) that the spirem breaks into a single and not a double chain of chromosomes. The possibility of finding a series of stages which really demonstrates this depends on the shape of the chromosomes in Oenothera. They are relatively short and thick, like many animal chromosomes, and quite unlike the twisted and tangled chromosomes of such forms as Lilium, whose relationships are so difficult to interpret. It is a curious fact that so many of the critical studies on reduction in plants have been made on forms with long narrow chromosomes, although many of the interpretations of critical stages can be made with much greater ease and certainty on forms having short and stout chromosomes.

In the paper just referred to, I concluded that the method was probably different in different genera, there being a side-by-side pairing of threads in some forms (parasynapsis),[6] but an end-to-end arrangement of the chromosomes to form a single spirem (telosynapsis)[6] in other forms. Subsequent papers by various investigators continue to describe both these methods, and the evidence in certain cases is so clear that I think there can remain no doubt that both these general methods occur in plants. MONTGOMERY concluded in 1898 (**26**) that there are different types of reduction in animals.

In a recent study of Fucus, YAMANOUCHI (**39**) shows clearly that at the time of reduction the ragged nuclear reticulum is gradually transformed into a single continuous thread, which enters into synapsis. This thread then becomes rearranged into regular loops converging to one side of the nucleus and forming the "bouquet" stage of EISEN (**6**). This stage is characteristic of many animals, in which it has been described by JANSSENS and DUMEZ (**17**), STEVENS (**34, 35**), MARÉCHAL (**25**), and JORDAN (**18**), to mention only a few. Each loop is composed of two chromosomes arranged end to end. Similarly the spirem in Fucus is made up of the maternal and paternal chromosomes arranged endwise in a single thread. This is what I have shown to be the case in Oenothera (**11**), although Oenothera has no typical "bouquet" stage, nor has any other angiosperm, so far as I am aware, though the second contraction phase, characteristic of various forms, probably corresponds to it. OVERTON (**30**) states that this stage does not occur in the plants with short chromosomes which he has examined, yet it occurs in Oenothera, in which the definitive chromosomes are very short and frequently almost globular. The second contraction phase is a well-marked stage of meiosis in Oenothera.

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[6] These convenient terms follow the usage of WILSON (36).

I may mention a few of the recent accounts involving an end-to-end arrangement of the chromosomes to form the spirem (telosynapsis). MOTTIER finds (**29**) that in Podophyllum, Lilium, and Tradescantia the two members of each bivalent chromosome were not side-by-side in the spirem, representing the halves of the longitudinally split thread, but were arranged end-to-end in the chromatic thread. LEWIS (**22**), in a study of Pinus and Thuja, finds no pairing of threads, but cross-segmentation of a single post-synaptic spirem to form the chromosomes. Just what relation his *fig. 15*, which indicates a reticulum as occurring about the time of chromosome formation, bears to the other stages, it is hard to say. LEWIS ventures the opinion that the second division is probably qualitative, but with a manifest lack of evidence to support it. SCHAFFNER (**33**), in Agave, finds bodies which he believes are bivalent prochromosomes, and a single spirem which segments to form the twelve bivalent chromosomes.

Among very recent accounts of reduction which involve a longitudinal pairing of threads (parasynapsis) may be mentioned that of GRÉGOIRE (**16**), with figures involving the critical stages in Lilium, Osmunda, and Allium; YAMANOUCHI (**38**) in Nephrodium; OVER-

TON (**30**) in Thalictrum, Calycanthus, and Richardia. ALLEN'S extensive earlier paper on Lilium (**1**) should also be mentioned. Although, in such cases as Lilium, both accounts of reduction are given for the same form by different authors, yet the evidence from such forms as Nephrodium on the one hand, and Tradescantia, Oenothera, and Fucus on the other hand, makes it very difficult to deny that both methods occur. A comparison of a wide range of forms whose reduction phenomena show many differences, will doubtless lead finally to an explanation of the nature and meaning of these differences. It is very evident that the time has passed when all the accounts of reduction in plants can be brought under a single scheme. The task of the future will be to interpret the meaning of the differences observed in various forms. Are they matters merely of cell mechanics, or are they related to hereditary processes? It is not impossible that correlations will be found between the method of reduction in an organism and its type of hereditary behavior. In other words, the phenomena of reduction and the distribution of elements during meiosis may condition to some extent the hereditary behavior of a plant or animal. This is already known to be the case with sex in insects. But it does not seem worth while entering into such theoretical possibilities on the basis of our present knowledge.

The fact that in this Oenothera hybrid the number of chromosomes in the pollen mother cells is the sum of those which enter the fusion nucleus at the time of fertilization, is to me clear evidence that each chromosome in the germ cells is the genetic descendant of one of the chromosomes in the fertilized egg. The work of various investigators seems to have established the genetic continuity of chromosomes from generation to generation of individuals. The manner in which the chromosomes are distributed during reduction in this Oenothera hybrid clearly shows that they behave as individuals at this time. WILSON'S recent account of the supernumerary chromosomes in Metapodius (**37**), a genus of Hemiptera, shows certain points of resemblance to the condition in Oenothera. WILSON finds that certain chromosomes (the idiochromosomes) may be present in duplicate, or may even have several representatives in the cells of certain individuals, which nevertheless show no external differences, the number of chromosomes being fixed for any individual, but varying in different individuals from 21 to 28 according to the number of supernumerary chromosomes present. The distribution of the supernumeraries during spermatogenesis is also irregular, so that the number varies in the different sperm nuclei of an individual. Occasional irregularities in the distribution of idiochromosomes during reduction were observed in the form having 22 chromosomes, both idiochromosomes passing to the same cell. This is believed to be the origin of the variation in different individuals, the supernumeraries being merely duplicates of the idiochromosomes.

Similarly, I have observed occasional irregularities in the chromosome distribution during reduction in nearly all the mutants of Oenothera examined, and this doubtless accounts for the different numbers of chromosomes found in different individuals, the extra chromosomes being duplicates of others already present, and the presence of 20 chromosomes in one individual of *O. lata*×*O. gigas* being due to the absence of one chromosome from one of the germ cells which produced that individual.

MONTGOMERY (**27**) first proposed the theory that in synapsis homologous chromosomes of maternal and paternal origin pair with each other, and much subsequent work, especially with animal chromosomes, sustains that view. As I have already shown, pairing of Oenothera chromosomes frequently fails to take place, and this allows a chance for the irregularities in chromosome distribution which occur. BOVERI (**3**, p. 54) has suggested that when there is an absence of pairing in synapsis in any organism, its chromosomes are hereditarily equivalent. In Oenothera there is clearly a tendency to pair, but it is only partly carried out. I have already compared (**11**, p. 25) the behavior of the chromosomes in this hybrid with the condition described by ROSENBERG (**31**) in the Drosera hybrid having 10+20 chromosomes. The method of segregation of chromosomes in the heterotypic mitosis is evidently different in the two hybrids, and this was used as an argument in favor of a different method of reduction in the two cases. In the Oenothera hybrid the 10–11 segregation shows that the segregation cannot be between chromosomes of maternal and paternal origin, but it must be remembered in this connection that the 14 paternal chromosomes are very probably a double set of 7 *O. Lamarckiana* chromosomes. The regularity with which the 10–11 segregation takes place, indicates that it is not merely a matter of chance, but that some mechanism, perhaps connected with the spindle, determines this regularity. A study of later generations of this hybrid should throw much light on the question whether the chromosomes of Oenothera are really unlike.

MORGAN (**28**) has recently shown that in certain phylloxerans, in which a generation of winged individuals produces (parthenogenetically) sexual males and females, the eggs are of two kinds, the female-producing eggs being large and the male-producing eggs small. Further, the females thus produced have the same number of chromosomes as the parthenogenetic females, while the males have two chromosomes less. Thus, in the formation of the polar body of the male egg, two extra chromosomes are extruded, so that the somatic cells of the male contain two less chromosomes than those of the female. Evidently there is here some sex-determining factor which antedates the chromosomal differences with the two kinds of eggs, and yet the chromosomes are the instruments of this factor, for the extrusion of the two chromosomes always precedes the development of the male individual. Studies of this sort will doubtless give us clearer notions regarding the respective rôles of chromosomes and cytoplasm in heredity.

In a former paper (**11**) I suggested that if the chromosomes of Oenothera are unlike in their hereditary capacities, then the occasional irregularities I have described in the chromosome distributions on the heterotypic spindle would furnish a possible basis for the appearance of a series of types, such as the mutants of *O. Lamarckiana*. I have since studied the reduction phenomena in *O. biennis* and other types, an account of which will be published later. *O. biennis*, in at least some of its geographical races, appears to be stable under ordinary conditions, though MACDOUGAL (**24a**) has obtained atypical forms by injections into its ovaries. The loose and frequently unpaired arrangement of the chromosomes in the central region of the heterotypic spindle is evidently a delicately adjusted condition which can easily be thrown out of balance. Yet in some way the balance is ordinarily maintained and is only occasionally deranged in such a way that a different chromosome distribution results. It is not impossible that the condition of mutation in *O. Lamarckiana* has arisen through a disturbance by some means of this delicate condition of balance.

But it is useless to speculate on such possibilities until it is known whether the chromosomes of Oenothera are really unlike, and at present there is no evidence in favor of this view except the inferential evidence from chromosomes in general. The fact that chromosomes reappear with each mitosis, showing the same differences where visible differences exist (except in the cases of amitosis, whose status in relation to hereditary processes is not at present understood), would seem to favor the assumption that they maintain their identity and are unlike. But I need not cite here the general arguments in favor of this hypothesis. The more definite evidence, such as that of the sex chromosomes in insects, is not necessarily of universal application.

SUMMARY

1. *O. lata*×*O. gigas* has 21 chromosomes in its somatic cells, 7 of maternal origin (*O. lata*) and 14 of paternal origin (*O. gigas*). In one individual the number was 20, owing probably to the absence of one chromosome from one of the germ cells which produced this individual.

2. These chromosomes segregate at the time of reduction, so that in individuals having 21 chromosomes half the germ cells receive 10 and half 11 chromosomes. In the individual having 20 chromosomes, 10 enter each germ cell. Occasionally one chromosome goes to the wrong pole of the spindle, so that in plants having 21 chromosomes a few germ cells are found having 9 or 12 chromosomes and in the plant with 20 chromosomes, occasional germ cells have 9 or 11 chromosomes. This irregularity in chromosome distribution accounts for the fact that different individuals in a race in some cases have different numbers of chromosomes.

3. The 10–11 segregation of chromosomes in the formation of the germ cells of this hybrid shows that there is not here a pairing and separation of homologous chromosomes of maternal and paternal origin, but that the segregation tends to be into two numerically equal groups.

4. Evidence from this and other work shows that there are two general methods of chromosome reduction in plants, one involving

a side-by-side pairing of chromatin threads (parasynapsis) to form a double spirem; the other involving an end-to-end arrangement (telosynapsis) of the maternal and paternal chromosomes to form a single spirem, which may afterward split longitudinally.

5. The behavior of the chromosomes in Oenothera supports the view of their genetic continuity from generation to generation, the number present in any individual being always the sum of the chromosomes in the germ cells from which that individual was formed.

6. If the chromosomes of Oenothera are unlike in their hereditary capacities their behavior furnishes a not improbable basis for the phenomena of mutation in *Oenothera Lamarckiana*. But it remains to be proven that the chromosomes of Oenothera are of unequal hereditary value.

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LITERATURE CITED

1. ALLEN, C. E., Nuclear division in the pollen mother cells of *Lilium canadense*. Annals of Botany **19**: 189–258. *pls. 6–9*. 1905.
2. BERGHS, J., Le fuseau hétérotypique de *Paris quadrifolia*. La Cellule **22**: 203–214. *pls. 2*. 1905.
3. BOVERI, T., Ergebnisse über die Konstitution der chromatischen Substanz des Zellkerns. pp. 130. *figs. 75*. Jena. 1904.
4. ———, Zellen Studien V. Ueber die Abhängigkeit der Kerngrösse und Zellenzahl des Seeigel-Larven von der Chromosomenzahl der Ausgangszellen. pp. 80. *pls. 2*. *figs. 7*. Jena.
5. DEVRIES, H., Bastarde von *Oenothera gigas*. Ber. Deutsch. Bot. Gesells. **26a**: 754–762. 1908.
6. EISEN, G., Spermatogenesis of Batrachoseps. Journ. Morph. **17**: 1–117. *pls. 14*. 1900.
7. ERDMANN, R., Experimentelle Untersuchung der Massenverhältnisse von Plasma, Kern, und Chromosomen in dem sich entwickelnden Seeigelei. Arch. Zellforsch. **1**: 76–136. 1908.
8. GATES, R. R., Pollen development in hybrids of *Oenothera lata* × *O. Lamarckiana*, and its relation to mutation. BOT. GAZETTE **43**: 81–115. *pls. 2–4*. 1907.
9. ———, Hybridization and germ cells of Oenothera mutants. BOT. GAZETTE **44**: 1–21. *figs. 3*. 1907.
10. ———, The chromosomes of Oenothera. Science N. S. **27**: 193–195. 1908.
11. ———, A study of reduction in *Oenothera rubrinervis*. BOT. GAZETTE **46**: 1–34. *pls. 1–3*. 1908.
12. GEERTS, J. M., Beiträge zur Kenntnis der cytologischen Entwicklung von *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. **26a**: 608–614. 1908

13. Geerts, J. M., Beiträge zur Kenntnis der Cytologie und der partiellen Sterilität von *Oenothera Lamarckiana*. Recueil Trav. Bot. Néerl. **5**:93–208. *pls. 5–22.* 1909.
14. Grégoire, V., et Wygaerts, A., La reconstruction du noyau et la formation des chromosomes dans les cinèses somatiques. La Cellule **21**:7–76. *pls. 1–2.* 1903.
15. Grégoire, V., La structure de l'élément chromosomique au repos et en division dans les cellules végétales (racines d'Allium). La Cellule **23**:311–353. *figs. 3. pls. 2.* 1906.
16. ———, La formation des gemini hétérotypique dans les végétaux. La Cellule **24**:369–420. *pls. 2.* 1907.
17. Janssens, F. A., et Dumez, R., L'élément nucléinien pendant les cinèses de maturation des spermatocytes chez *Batrachoseps attenuatus* et *Pletodon cinereus*. La Cellule **20**:421–460. *pls. 5.* 1903.
18. Jordan, H. E., The spermatogenesis of *Aplopus Mayeri.* Carnegie Institution Publ. 102. pp. 13–36. *pls. 1–5.* 1908.
19. Lawson, A. A., Origin of the cones of the multipolar spindle in Gladiolus. Bot. Gazette **30**:145–153. *pl. 12.* 1900.
20. ———, On the relationships of the nuclear membrane to the protoplast. Bot. Gazette **35**:305–319. *pls. 15.* 1903.
21. ———, Studies in spindle formation. Bot. Gazette **36**:81–100. *pls. 15, 16.* 1904.
22. Lewis, I. M., The behavior of the chromosomes in Pinus and Thuja. Annals of Botany **22**:529–556. *pls. 29, 30.* 1908.
23. Lillie, F. R., Observations and experiments concerning the elementary phenomena of embryonic development in Chaetopterus. Journ. Exp. Zool. **3**:153–268. *pl. 1. figs. 78.* 1906.
24. Lutz, Anne M., Notes on the first generation hybrid of *Oenothera lata* × *O. gigas.* Science N. S. **29**:263–267. 1909.
24a. MacDougal, D. T., Vail, A. M., and Shull, G. H., Mutations, variations, and relationships of the Oenotheras. Carnegie Institution Publ. 81. pp. 92. *pls. 22. figs. 73.* 1907.
25. Maréchal, J., Sur l'ovogénèse des Sélaciens et de quelques autres Chordates. I. La Cellule **24**:7–239. *pls. 1–11.* 1907.
26. Montgomery, T. H., The spermatogenesis in Pentatoma up to the formation of the spermatid. Zool. Jahrb. Anat. Ont. **12**:1–88. *pls. 1–5.* 1898.
27. ———, A study of the chromosomes of the germ cells of Metazoa. Trans. Amer. Phil. Soc. **20**:154–236. *pls. 4–8.* 1901.
28. Morgan, T. H., Sex determination and parthenogenesis in phylloxerans and aphids. Science N. S. **29**:234–237. 1909.
29. Mottier, David M., The development of the heterotypic chromosomes in pollen mother cells. Annals of Botany **21**:309–347. *pls. 27, 28.* 1907.
30. Overton, J. B. On the organization of the nuclei in the pollen mother cells of certain plants with especial reference to the permanence of the chromosomes. Annals of Botany **23**:19–61. *pls. 1–3.* 1909.

31. ROSENBERG, O., Ueber die Tetradentheilung eines Drosera-Bastardes. Ber. Deutsch. Bot. Gesells. **22**:47-53. *pls. 4.* 1904.

32. ———, Cytological studies on the apogamy in Hieracium. Botanisk Tidsskrift **28**:143-170. *figs. 13. pls. 1, 2.* 1907.

33. SCHAFFNER, JOHN H., The reduction division in the microsporocytes of *Agave virginica.* BOT. GAZETTE **47**:190-214. *pls. 12-14.* 1909.

34. STEVENS, N. M., Studies in spermatogenesis, with special reference to the accessory chromosome. Carnegie Institution Publ. 36.[1] pp. 32. *pls. 7.* 1908.

35. ———, A comparative study of the heterochromosomes in certain species of Coleoptera, Hemiptera, and Lepidoptera, with especial reference to sex determination. Carnegie Institution Publ. 36.[2] pp. 33-74. *pls. 8-15.* 1906.

36. WILSON, E. B., Studies on chromosomes. IV. Journ. Exp. Zool. **6**:84. 1909.

37. ———, Studies on chromosomes. V. The chromosomes of Metapodius. Journ. Exp. Zool. **6**:147-205. *pl. 1. figs. 13.* 1909.

38. YAMANOUCHI, SHIGÉO, Sporogenesis in Nephrodium. BOT. GAZETTE **45**:1-30. *pls. 1-4.* 1908.

39. ———, Mitosis in Fucus. BOT. GAZETTE **47**:173-197. *pls. 2-11.* 1909.

EXPLANATION OF PLATES XII-XIV

The figures were drawn with the aid of a Bausch & Lomb camera and a Zeiss apochromatic objective 2^{mm}, ap. 1.30, particular care being taken to represent the chromosomes accurately. The figures in plate XII were drawn with a Zeiss compensating ocular 12, those of plates XIII and XIV with ocular 18. All are reduced one-fourth in reproduction, which leaves the figures in plates XIII and XIV magnified as in my last paper (**11**) on reduction.

PLATE XII

All figures from the plant having 20 chromosomes.

FIG. 1.—Early telophase of the heterotypic mitosis in the pollen mother cells before formation of the nuclear membrane; 10 chromosomes, all clearly bivalent; in many the halves are dumb-bell shape.

FIG. 2.—Same stage; 11 chromosomes, many irregular; 9 chromosomes at the opposite end of this spindle; dark staining bodies in periphery of cytoplasm.

FIG. 3.—Telophase, showing 10 chromosomes of various shapes.

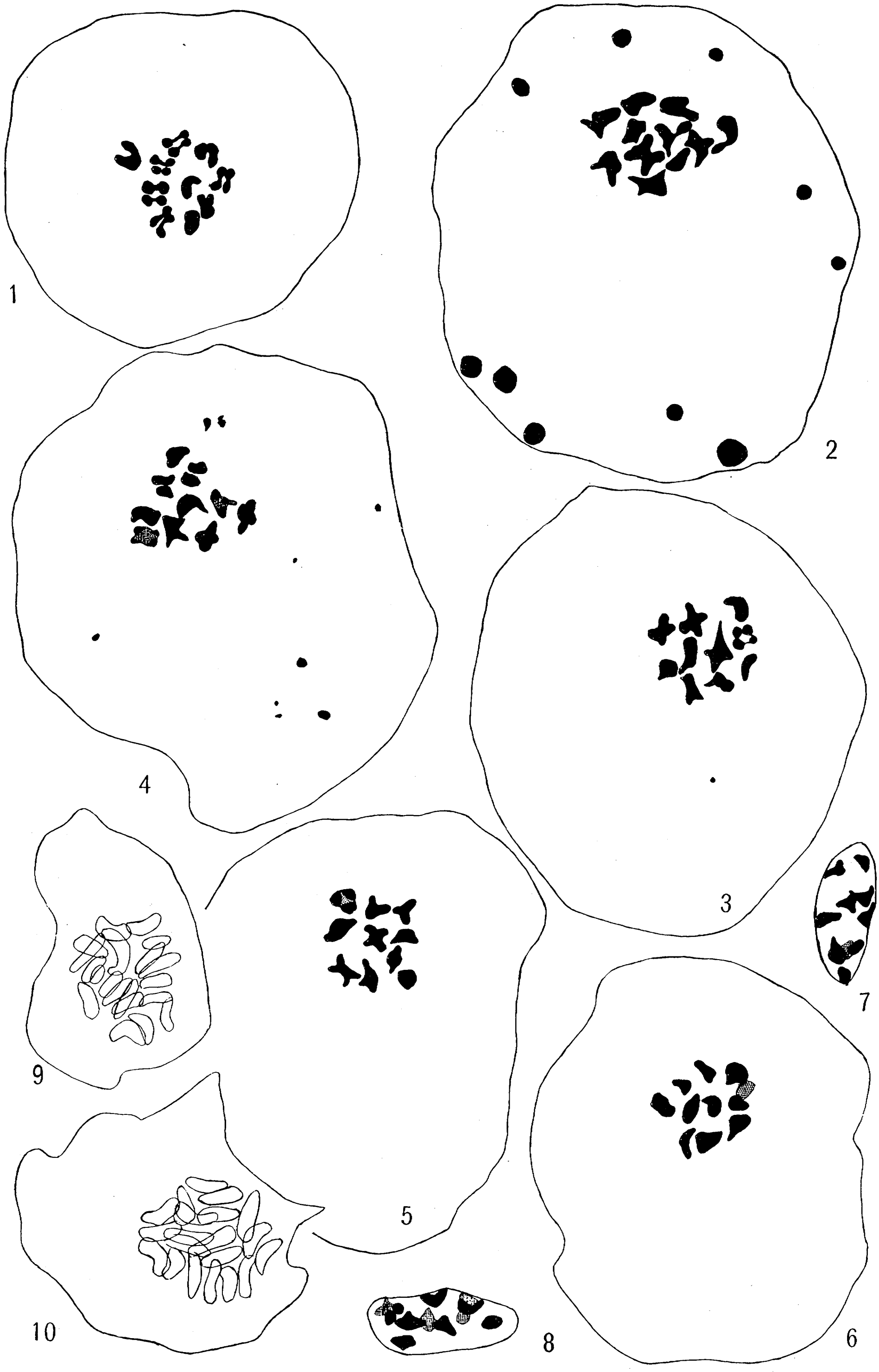
FIG. 4.—Telophase, showing 10 chromosomes, most of them clearly bivalents.

FIG. 5.—Same as *fig. 4.*

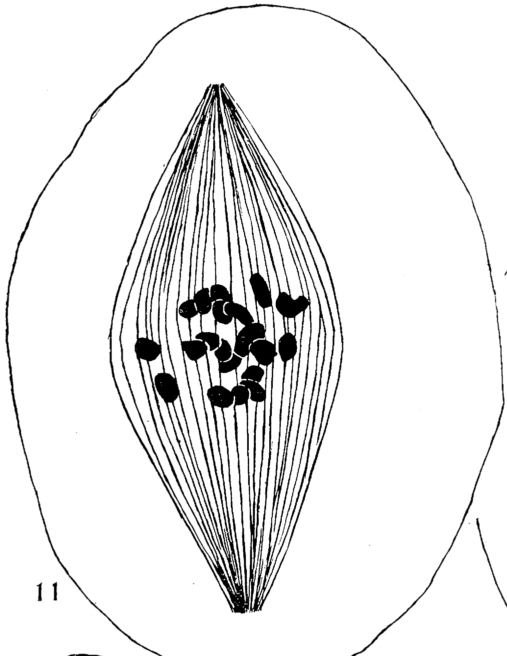
FIG. 6.—Telophase, showing 11 chromosomes, with no indication of their bivalent character; 9 at opposite end of spindle; apparent size of chromosomes in all figures varies to a certain extent according to the depth of stain.

FIGS. 7, 8.—Later telophases, soon after formation of nuclear membrane, each showing 10 chromosomes.

FIGS. 9, 10.—Metaphase groups of chromosomes in somatic cells from middle layers of anther wall, each showing 20 chromosomes; indications of an arrangement of the chromosomes in pairs.



GATES on CHROMOSOMES of OENOTHERA



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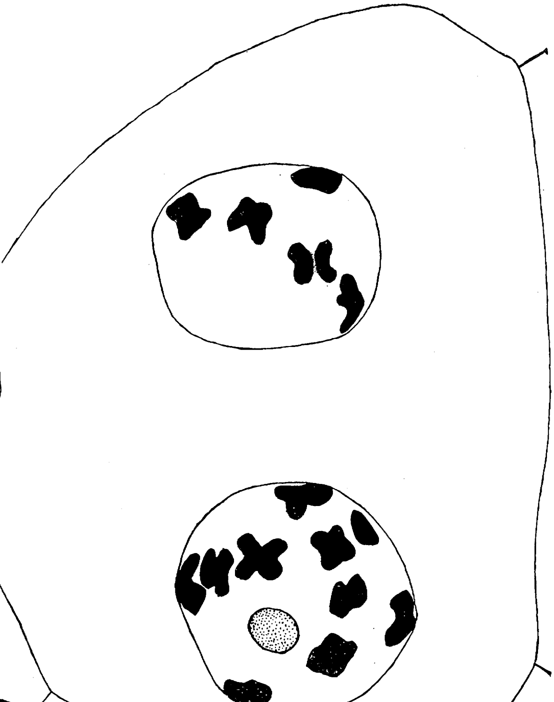

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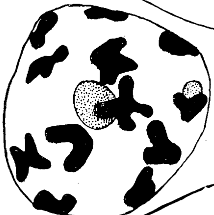

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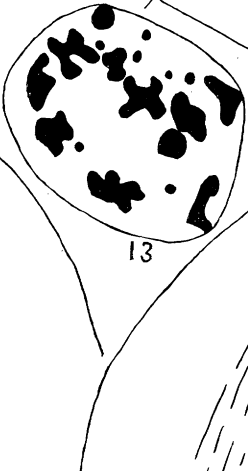

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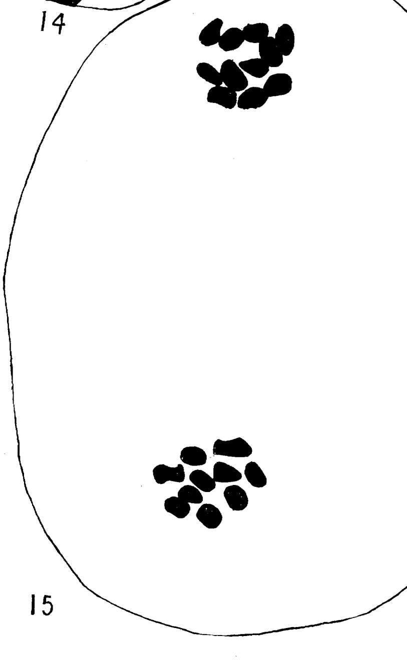

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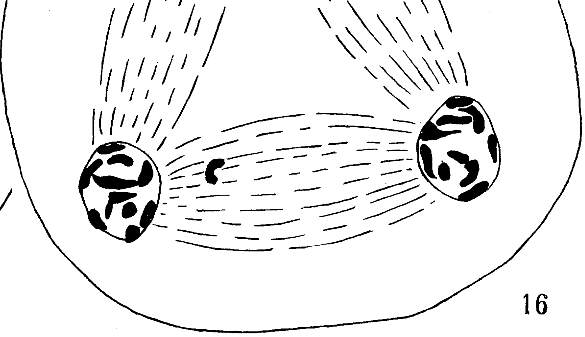

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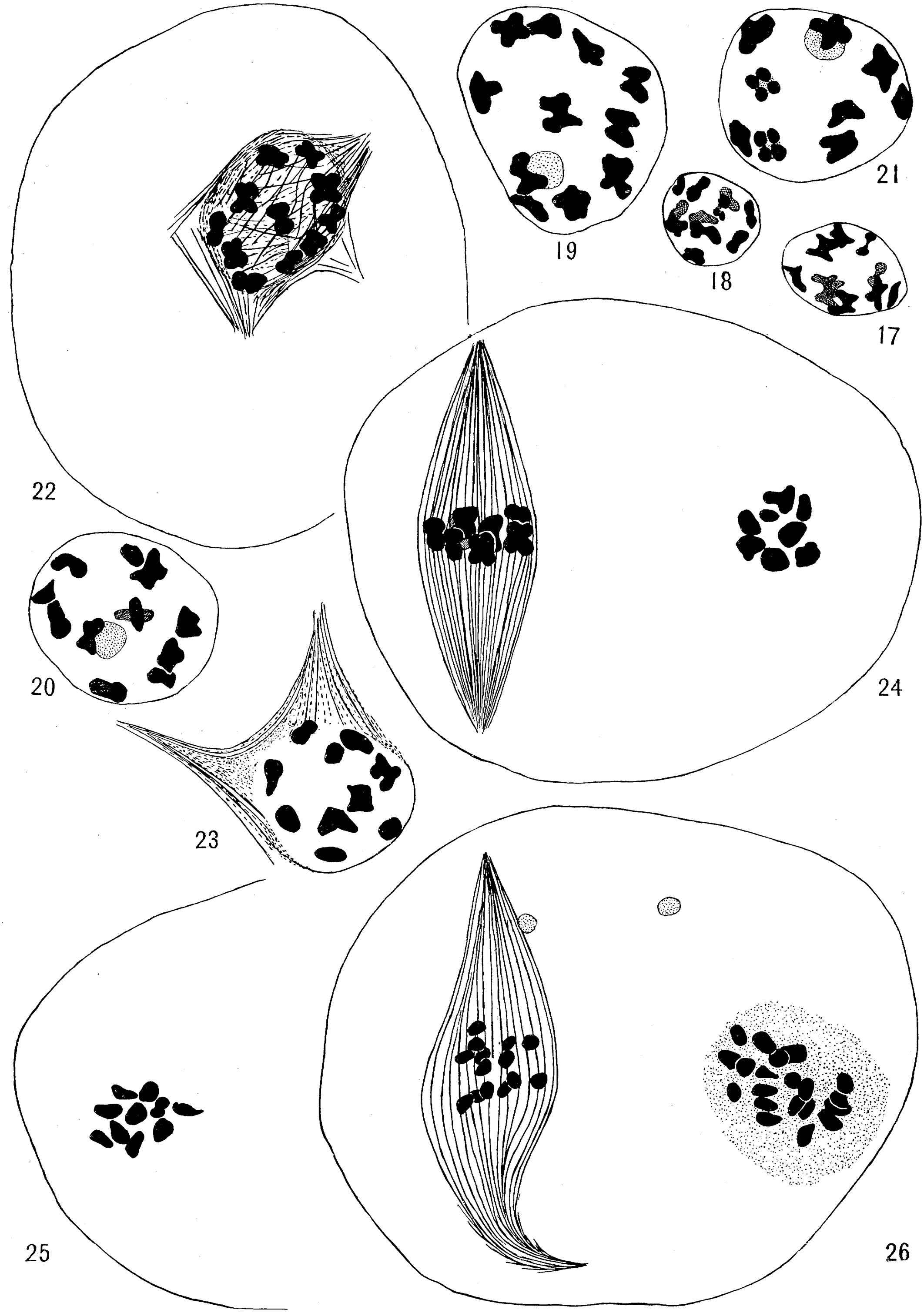
GATES on CHROMOSOMES of OENOTHERA



GATES on CHROMOSOMES of OENOTHERA

PLATE XIII

Figures in plates XIII and XIV from plants having 21 chromosomes; not numbered in developmental order.

FIG. 11.—Heterotypic spindle in side view, showing 20 or 21 chromosomes, not forming an equatorial plate, but loosely aggregated in the median region of the spindle; cf. *fig. 15*.

FIG. 12.—Telophase of heteroptypic mitosis, showing both daughter nuclei; one, uncut, shows 10 chromosomes.

FIG. 13.—Telophase, showing 11 chromosomes and a number of very small nucleoli; an unusual condition, probably due to the failure of the nucleoli to fuse at an earlier stage.

FIG. 14.—Telophase, showing 12 bivalent chromosomes, lying closely against the wall, tipped at various angles to the plane of view, which gives them a variety of appearances; an exceptional case, in which one chromosome too many has passed to the end of the spindle; 9 at opposite end; 2 nucleoli.

FIG. 15.—Metaphase of homotypic mitosis in polar view, showing equatorial plates of chromosomes, 10 chromosomes on one spindle and 11 on the other; cf. *fig. 11*.

PLATE XIV

FIG. 16.—Telophase of homotypic mitosis, showing three daughter nuclei; one chromosome left behind in the cytoplasm, leaving 10 chromosomes each in two of the daughter nuclei; many chromosomes show characteristic dumb-bell shape.

FIGS. 17, 18.—Very early telophase of the heterotypic mitosis, just after the nuclear membrane has been formed around the daughter nuclei; one shows 10 bivalent chromosomes, the other 11.

FIGS. 19, 20.—Telophases, each showing 11 bivalent chromosomes.

FIG. 21.—Telophase with 9 chromosomes only.

FIG. 22.—Prophase of the homotypic mitosis, showing 11 bivalent chromosomes, which have the same appearance as in the previous telophase; nuclear membrane just broken down, its position still occupied by a weft of fibrillae, against which the chromosomes lie; cones of the multipolar spindle forming.

FIG. 23.—Same stage as *fig. 22;* a less common condition, in which spindle formation begins on one side of the nucleus; 10 chromosomes, only a few of which show bivalent character.

FIG. 24.—Metaphase of the homotypic mitosis, showing one spindle in side view and the other in polar view; chromosomes not all shown.

FIG. 25.—Equatorial plate of homotypic spindle, showing 10 chromosomes in a single plane, few showing their bivalent character.

FIG. 26.—Early anaphase of homotypic mitosis, after division of the chromosomes; one spindle in side view, showing only part of the chromosomes (probably 20 in all); the other spindle in polar view, showing 20 chromosomes (two more are on this spindle in the next section); 2 nucleoli in the cytoplasm near the spindles.